

Title: Geotaxis Baseline Data for Drosophila

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Abstract

Geotaxis profiles for 20 Drosophila species and semispecies at different ages have been examined using a calibrated, adjustable slant board device. Measurements were taken at 5° intervals ranging from 0° to 85°. Clear strain and species differences are observed, with some groups tending to move upward (- geotaxis) with increasing angles, while others move downward (+ geotaxis). Geotactic responses change with age in some, but not all experimental groups. Sample geotaxis profiles are presented and their application to ecological and aging studies are discussed. Data provide a baseline for future evaluations of the biological effects of microgravity.

Introduction

Geotaxis is defined as directed movement mediated by gravity. Since the early twentieth century, geotaxis in insects (Drosophila in particular) has been used as a model system for investigating a diversity of biological problems including:

1. effects of environmental cues on behavior (Carpenter, 1905),
2. receptor function and mechanisms of sensory systems (Horn, 1975),
3. genetic architectures underlying behavior (Hirsch, 1959; Dobzhansky and Spassky, 1962; Walton, 1968; Woolf et al., 1978),
4. learning capabilities in insects (Murphey, 1969),
5. behavioral bases for ecological differences among species (Fogleman and Markow, 1982) and between life stages of the same species (Markow, 1979),
6. aging processes and senescence (Herman et al., 1971; Miquel et al., 1976; 1979; Leffelaar and Grigliatti, 1984a,b).

In studies of aging, changes in geotactic response have provided biological markers for:

- a) identifying aging individuals,
- b) examining the effects of environmental factors on aging individuals,
- c) determining the physiological basis of behavior loss in aging individuals,
- d) localizing changes at the cellular and molecular levels that accompany such behavior losses,
- e) identifying genetic mutants that alter aging patterns.

Despite the broad array of questions that can be addressed using Drosophila geotaxis in experimental paradigms, problems in the interpretation of results may become evident. It is known, for example, that geotactic response can be a function of the apparatus being utilized, and that different components of the response may be reflected by each experimental design (Murphey and Hall, 1969; Grossfield, 1978; Levine et al., 1981). Therefore, species, strain and age comparisons of geotactic responses remain tenuous as long as different laboratories measure geotaxis in different ways.

The present series of experiments represents the most extensive body of work known which has utilized identical experimental methodology for all subjects. Direct interspecific comparisons can be made among 20 species and semispecies, strains can be compared within many of these groups, and age comparisons can be compared within many of these groups, and age comparisons can be carried out for all. Additionally, direct comparisons are now possible between flies representing different degrees of phylogenetic relatedness, as well as between flies representing different ecological backgrounds (see Table 1).

Methods and Materials

Animals and Housing

Drosophila species, semispecies, strains and test ages are listed in Table 1. All flies were reared on a raisin-based culture medium at 19-20C, 67%RH and constant light.

Experimental Procedures

Flies aged 1-2 days, 7-9 days and 30-36 days were tested separately in the rearing chamber. A test lasting 62.5 min began after 20 individuals were aspirated into the center of a 30cm glass tube marked on the outside into three equal sections. The tube was placed on an adjustable board calibrated for angles ranging from 0° to 85° . A maximum of nine tubes were tested concurrently. Starting at 0° and ending at 85° the board was raised 5° every three minutes. After each three minute interval, the number of flies in each third of the tube was counted. Additionally, each time before the board was raised, it was held at 5° and dropped in order to dislodge flies from their previous position, and then raised to 45° so that flies would start from the bottom when the board was set at the new test angle. This was done because pilot work indicated that a) when flies were not dislodged each time, they would often distribute themselves in the tube and remain motionless for much of the test period, and b) after being dislodged, those flies raised to steep angles would slide to the bottom of the tube while those raised to less severe angles did not. 30 replicate tests were carried out for each experiment.

This test procedure provides information about the minimum angle required to elicit a geotactic response (geotactic sensitivity), and the effects of 18 experimental angles on the tendency to move in a particular direction (geotaxis profile). Measurements can be taken in ascending, descending or random order of presentation of angles.

Analysis of Data

Intraspecific. The mean number of flies (\pm S.D.) in each section of the tube at all 18 experimental angles was calculated for each species, strain and age group (based on 30 replicates of 20 flies each). At each experimental angle, the mean number of flies in the top and bottom sections of the tube were compared using the Chi Square test ($df=1$) with the Yates correction for continuity (Zar, 1974). Angles at which these means became significantly different were recorded. Using this method, age groups and/or strains can be compared at any angle or set of angles.

Interspecific. The mean number of flies in the top or bottom of the tube can be compared among any two or more species at any experimental angle using Student's *t*-test. Thus, species differences at any age can be directly compared.

Table 1. Drosophila Subjects Tested For Geotactic Sensitivity and Geotaxis Profiles.

| <u>Subgenus</u> | <u>Species Group</u> | <u>Species (# strains)</u> | <u>Ecological Background (Throckmorton, 1975)</u> | <u>Ages Tested (days)</u> |
|-------------------|----------------------|---|--|--|
| <u>Drosophila</u> | Funebris | funebris (1) | cosmopolitan | 1-2, 7-9, 30-36 |
| | Immigrans | immigrans (1) | cosmopolitan | 1-2, 7-9 |
| | Repleta | arizonensis (3) | desert | 1-2, 7-9, 30-36 |
| | | mojavensis (3) | desert | 1-2, 7-9, 30-36 |
| | | mulleri (1) | desert | 1-2, 7-9, 30-36 |
| | Robusta | robusta (1) | temperate forest | 1-2, 7-9, 30-36 |
| <u>Sophophora</u> | Virilis | virilis (1) | temperate forest | 1-2, 7-9, 30-36 |
| | | americana (1) | temperate forest | 1-2, 7-9, 30-36 |
| | | montana (1) | temperate forest | 1-2, 7-9, 30-36 |
| | | | | |
| | Melanogaster | melanogaster (2) | cosmopolitan | 1-2, 7-9, 30-36 |
| | | simulans (2) | cosmopolitan | 1-2, 7-9, 30-36 |
| | | ananassae (1) | cosmopolitan | 1-2, 7-9, 30-36 |
| | | takahashii (1) | subtropical forest | 1-2, 7-9, 30-36 |
| | Obscura | obscura (1) | temperate forest | 1-2, 7-9, 30-36 |
| | | pseudoobscura (1) | temperate forest | 1-2, 7-9, 30-36 |
| | | miranda (1) | temperate forest | 1-2, 7-9, 30-36 |
| | Willistoni | paulistorum (1) *Amazonian (1) *Interior (1) *Transitional (1) *Centroamericana (2) | tropical forest tropical forest tropical forest tropical forest | 1-2, 7-9, 30-36 1-2, 7-9, 30-36 1-2, 7-9, 30-36 1-2, 7-9, 30-36 |

* semispecies

GT Ratio. In order to facilitate comparisons, results from all experimental groups were quantified and graphically described using a ratio introduced by Bean (1977). GT is a dimensionless ratio expressing the proportion of the half-tube population of flies that has undergone a net redistribution between halves of the experimental tube at each angle. The ratio takes on values from -1 to +1, where the sign corresponds to the direction of movement. Negative values reflect net upward movement of flies (negative geotaxis represents movement away from the force of gravity). Since calculation of the GT ratio requires the number of flies in each half of the tube, the flies observed in the middle third of the experimental tube were equally divided between top and bottom halves.

Results and Discussion

Figs. 1 and 2 present a sample of geotaxis profiles and how such data can be applied to a variety of biological questions.

Ecological Studies

Fig. 1a reflects the geotaxis profiles just after eclosion (1-2 days of age) for three desert species, D. arizonensis, D. mojavensis and D. mulleri. D. arizonensis and D. mojavensis are closely related sibling species that feed and breed on columnar cacti, whereas D. mulleri is found on the lower growing prickly pear cactus, Opuntia (Zouros, 1973). D. mulleri shows a clear positive geotactic tendency which corresponds to its preference for the ground-dwelling Opuntia. By contrast, the negative geotaxis of D. arizonensis would enable it to utilize its primary resources growing higher. The tendency of D. mojavensis to show neither preference may reflect a means of reducing competition with its close relative, D. arizonensis, when they occupy the same resources.

Fig. 1b reflects the geotaxis profiles of three tropical D. paulistorum semispecies, Transitional, Amazonian and Interior. The distributions and temperature capabilities of Amazonian and Interior often overlap, whereas those of Transitional are quite different (Schnebel and Grossfield, 1984, 1986a,b). Since Amazonian and Transitional are rarely found together, their similar geotactic tendencies are not expected to create situations of resource competition. However, the potential for competition between Amazonian and Interior is great, and their different geotaxis profiles represent a mechanism for vertically partitioning resources.

Aging Studies

D. melanogaster is the most commonly used species other than humans in studies of aging and senescence. Figs. 2a,b show the geotaxis profiles of two strains of D. melanogaster as individuals grow older. The Florida strain (mel-F) appears to lose its negative geotactic tendency by the third test age (30-36 days). The Oregon-R strain (+/+) shows a similar change, but the loss of geotactic response already occurs by the second test age (7-9 days). Structural comparisons of the two strains at critical ages could reveal underlying differences contributing to such age effects. A complementary approach in which D. melanogaster strains are compared with closely related species showing no effects of aging on geotaxis (Fig. 2c: D. simulans) could prove equally informative.

In addition, since the genetics of D. melanogaster are well understood and its chromosomes have been mapped, the search for mutants affecting geotaxis in these strains may be profitable in analyzing the genetic and molecular bases of aging processes influencing this system.

Fig. 1. Sample Geotaxis Profiles: Ecological Studies.

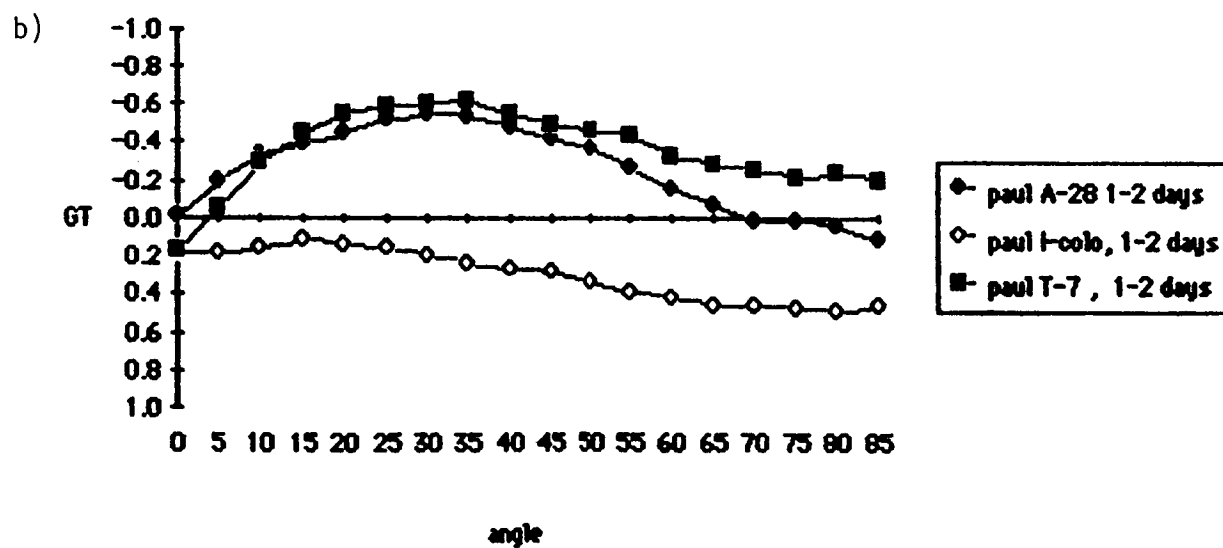
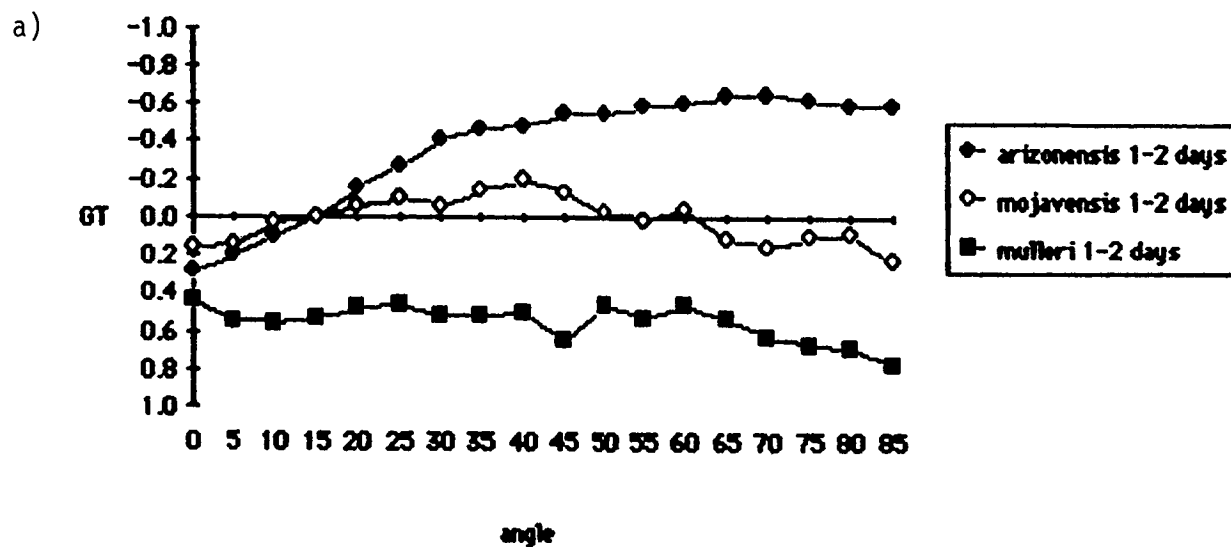
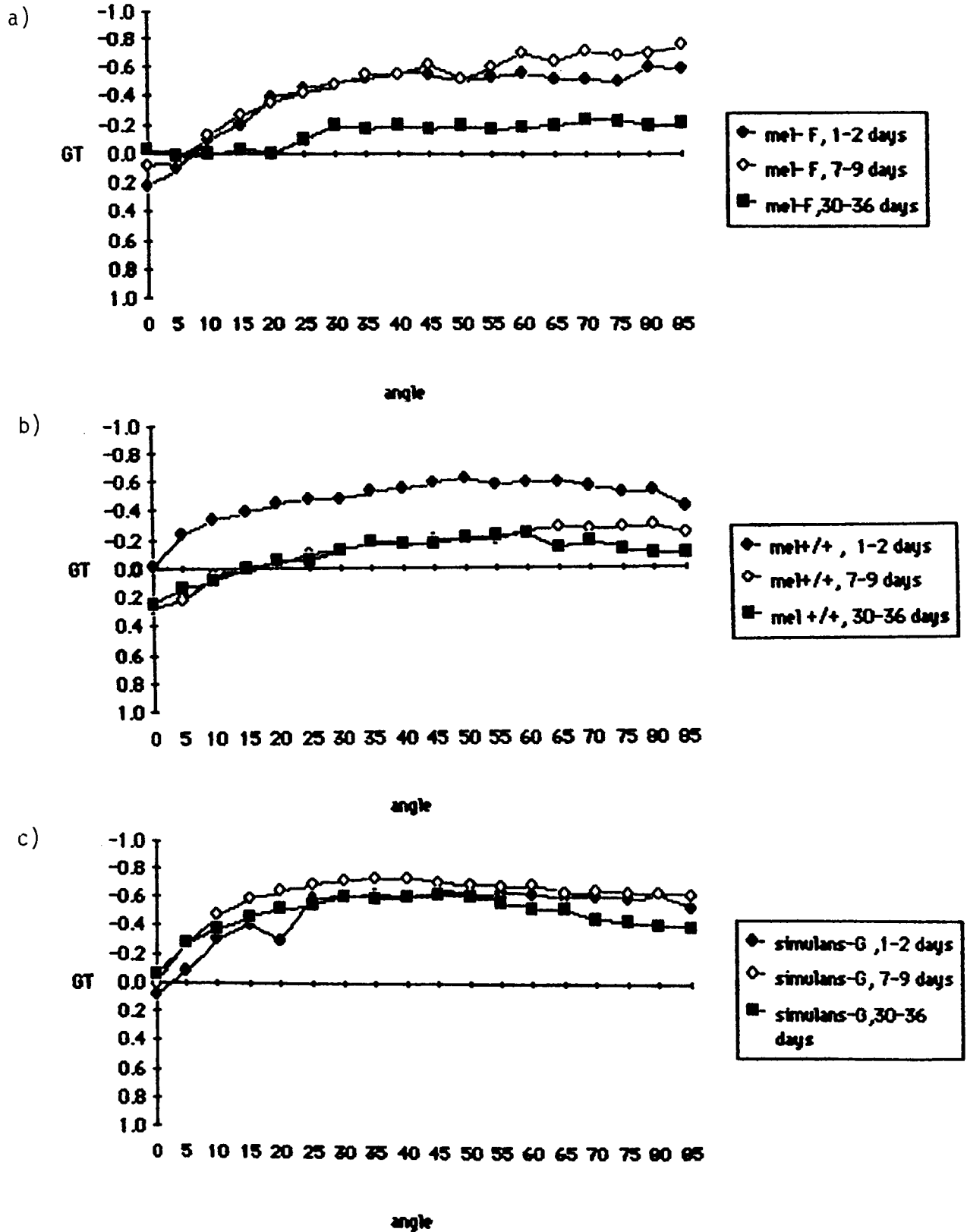


Fig. 2. Sample Geotaxis Profiles: Aging Studies.



These data demonstrate the applicability of the Drosophila geotaxis system for addressing questions of biological importance. The broad range of subjects investigated here under identical experimental conditions provides a foundation for direct systematic comparisons among different species, strains and age groups. These attributes make the present work invaluable for providing baseline data that can be used in future evaluations of the biological effects of microgravity.

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References

- Bean, B. 1977. Geotactic behavior of Chlamydomonas. J. Protozool. 24:394-401.
- Carpenter, F.W. 1905. The reactions of the pomace fly (Drosophila ampelophila Loew) to light, gravity and mechanical stimulation. Amer. Natur. 39:157-171.
- Dobzhansky, Th. and B. Spassky. 1962. Selection for geotaxis in monomorphic and polymorphic populations of Drosophila pseudoobscura. Proc. Nat. Acad. Sci. 48:1704-1712.
- Fogleman, J.C. and T.A. Markow. 1982. Behavioral differentiation between two species of cactophilic Drosophila. II. Pupation site preference. Southw. Natur. 27:315-320.
- Grossfield, J. 1978. Non-sexual behavior in Drosophila. In: The Genetics and Biology of Drosophila, Vol. 2b. Ashburner, M. and T.R.F. Wright (Eds.), p. 1-126. Academic Press: London.
- Herman, M.M., J. Miquel and M. Johnson. 1971. Insect brain as a model for the study of aging. Age-related changes in Drosophila melanogaster. Acta Neuropath. 19:167-183.
- Hirsch, J. 1959. Studies in experimental behavior genetics: II. Individual differences in geotaxis as a function of the chromosome variations in synthesized Drosophila populations. J. Comp. Physiol. Psych. 52:304-308.
- Horn, E. 1975. The contribution of different receptors to gravity orientation in insects. Fortschr. Zool. 23:1-20.
- Leffelaar, D. and T. Grigliatti. 1984a. Age-dependent behavior loss in adult Drosophila melanogaster. Dev. Genet. 4:211-217.
- _____. 1984b. A mutation in Drosophila that appears to accelerate aging. Dev. Genet. 4:199-210.
- Levine, L., S. Kessler and R.F. Rockwell. 1981. Components of maze running in Drosophila pseudoobscura and Drosophila persimilis. Behav. Genet. 11:415-423.
- Markow, T.A. 1979. A survey of intra- and interspecific variation for pupation height in Drosophila. Behav. Genet. 9:209-217.
- Miquel, J., A.C. Economos, K.G. Bensch, H. Atlan and J.E. Johnson, Jr. 1979. Review of cell aging in Drosophila and mouse. Age 2:78-88.
- Miquel, J., P.R. Lundgren, K.G. Bensch and H. Atlan. 1976. Effects of temperature on the life span, vitality and fine structure of Drosophila melanogaster. Mech. Ageing Dev. 5:347-370.
- Murphey, R.M. 1969. Spatial discrimination performance of Drosophila melanogaster: some controlled and uncontrolled correlates. Anim. Behav. 17:43-46.
- Murphey, R.M. and C.F. Hall. 1969. Some correlates of negative geotaxis in Drosophila melanogaster. Anim. Behav. 17:181-185.

- Schnebel, E.M. and J. Grossfield. 1984. Mating-temperature range in Drosophila. Evolution 38:1296-1307.
- _____. 1986a. Oviposition-temperature range in four Drosophila species triads from different ecological backgrounds. Amer. Midl. Natur. In press.
- _____. 1986b. Pupation-temperature range in 12 Drosophila species from different ecological backgrounds. Experientia 42:600-604.
- Throckmorton, L.H. 1975. The phylogeny, ecology and geography of Drosophila. In: Handbook of Genetics, Vol. III. King, R. (Ed.), p. 421-469. Plenum Press: New York.
- Walton, P.D. 1968. The genetics of geotaxis in Drosophila melanogaster. Can. J. Genet. Cytol. 10:673-687.
- Woolf, C.M., H.M. Sasmer and T.A. Markow. 1978. Positive and negative geotaxis: Sex-linked traits in Drosophila pseudoobscura. Behav. Genet. 8:65-71
- Zar, J.H. 1974. Biostatistical Analysis. Prentice Hall: Englewood Cliffs. 620p.
- Zouros, E. 1973. Genic differentiation associated with the early stages of speciation in the mulleri subgroup of Drosophila. Evolution 27:601-621.